

# Structure of staminate flowers, microsporogenesis, and microgametogenesis in *Helosis cayennensis* var. *cayennensis* (Balanophoraceae)

Ana María González\*, Orlando Fabián Popoff & Cristina Salgado Laurenti

Instituto de Botánica del Nordeste-IBONE-(UNNE-CONICET), Facultad de Ciencias Agrarias, Sarg. Cabral 2131, Corrientes, Argentina, CP 3400; anitama39@gmail.com; landypopoff@gmail.com; cristinalaurenti@yahoo.com

## Abstract

González, A.M., Popoff, O.F. & Salgado Laurenti, C. 2013. Structure of staminate flowers, microsporogenesis, and microgametogenesis in *Helosis cayennensis* (Balanophoraceae). *Anales Jard. Bot. Madrid* 70(2): 113-121.

We analyzed the microgametogenesis and microsporogenesis of the male flowers of the holoparasitic *Helosis cayennensis* (Sw.) Spreng. var. *cayennensis* using optical and scanning electron microscopy. The unisexual flowers are embedded in a dense mass of uniseriate trichomes (filariae). Male flowers have a tubular 3-lobed perianth, with bilayered and non vascularized tepals. The androecium consists of three stamens with filaments and thecae connated into a synandrium. It has adnate a free central pistilode without megagametophyte. Staminal filaments, fused at their base to the perianth tube and distally free along a short section, have a single vascular bundle. The distal portion of the synandrium is formed by nine pollen sacs: six outer sacs are located laterally to each filament and three longer inner sacs. The anther wall consists of the epidermis, two parietal layers (that collapse at anther maturity), and an uninucleate secretory tapetum. There is no endothecium. During microsporogenesis, the stem cells produce tetrads of microspores by meiosis. The cytokinesis is simultaneous, forming tetrahedrally arranged tetrads. When pollen grains are in the tricellular state, the synandrium emerges from the mass of filariae, and anthers dehiscence occurs apically through longitudinal slits. In conclusion, despite the extreme reduction of flowers, the anatomic characteristics and gametophyte development of staminate flowers of *H. cayennensis* are perfectly normal and functional. They are thus highly similar to other genera of the holoparasitic subfamily Helosidoideae. Sterile parts of flowers and inflorescence maintain the same distinctive and aberrant features of the plant vegetative parts.

**Keywords:** anther development, Balanophoraceae, *Helosis*, holoparasites, microgametogenesis, microsporogenesis, synandrium, unisexual flowers.

## INTRODUCTION

Within Balanophoraceae, *Helosis* Rich. is a monotypic genus of the Helosidoideae. This subfamily is characterized by an endogenous inflorescence with flowers embedded in a layer of filiform trichomes, and the connate stamens of the male flowers forming a trimerous synandrium (Eberwein, 2000; Nickrent, 2002).

*Helosis cayennensis* (Sw.) Spreng. var. *cayennensis* is a holoparasitic geophyte. Its vegetative body, or tuber, grows underground and produces rhizomes without buds or leaves (Kuijt, 1969; Mauseth & al., 1992; Hsiao & al., 1993). The only record of this genus in Argentina is from Apipé Grande Island (province of Corrientes) by Fontana & Popoff (2006),

## Resumen

González, A.M., Popoff, O.F. & Salgado Laurenti, C. 2013. Estructura de las flores estaminadas, microsporogénesis y microgametogénesis en *Helosis cayennensis* (Balanophoraceae). *Anales Jard. Bot. Madrid* 70(2): 113-121 (en inglés).

Se analizó la estructura de las flores masculinas de *Helosis cayennensis* (Sw.) Spreng. var. *cayennensis* con microscopía óptica y electrónica de barrido y se estudió la microsporogénesis y la microgametogénesis. Las flores funcionalmente unisexuales se encuentran embebidas en una densa capa de tricomas uniseriados. Las flores estaminadas presentan un perianto tubular, 3-lobado, con tépalos biestratificados y sin vascularización. Androceo formado por tres estambres con filamentos y tecas connadas en un sinandro. Las flores presentan un pistilodio central sin desarrollo de megagametofito. Los filamentos estaminales, con un solo haz vascular, están soldados próximalmente al tubo del perianto y hacia la parte distal son libres a lo largo de un corto trecho. La porción apical del sinandro está formada por nueve sacos polínicos: seis externos ubicados lateralmente en cada filamento y tres sacos internos de mayor longitud. La pared de la antera consta de epidermis, dos estratos parietales colapsados a la madurez de la antera y un tapetum secretor uninucleado. No posee endotecio. Durante la microsporogénesis las células madres de las micrósporas producen por meiosis tétradas de micrósporas, la citocinesis es simultánea y se forman tétradas de disposición tetraédrica. Cuando los granos de polen se encuentran en estado tricelular, el sinandro emerge de la masa de tricomas y la dehiscencia se produce por aberturas apicales longitudinales. Como conclusión se observó que a pesar de la extrema reducción de las flores, las características anatómicas y los procesos de esporogénesis y gametogénesis de las flores estaminadas de *H. cayennensis* son perfectamente normales y siguen patrones usuales, siendo muy similares a otros géneros de holoparásitas estudiadas de la subfamilia Helosidoideae. Las porciones estériles, tanto de las flores como de la inflorescencia, presentan las mismas características aberrantes ya descritas en el cuerpo vegetativo de esta especie.

**Palabras clave:** Balanophoraceae, desarrollo de antera, flores unisexuales, *Helosis*, holoparásitas, microsporogénesis, microgametogénesis, sinandro.

who highlight the similarity of *Helosis* inflorescences with fruiting bodies of certain fungi.

The only information of inflorescences and flowers of this genus are the morphological descriptions in taxonomic studies (Hansen, 1980b; Hansen & Engell, 1978; Martínez y Pérez & Rosas, 1995). Inflorescences are the only aerial portions of the plant to emerge endogenously from the rhizomes and are initially covered with a volva (Mauseth & al., 1992). The rest of the volva tissue remains at the base of the inflorescence and allows differentiation between *H. cayennensis* and *H. mexicana* (Liebm) B. Hansen varieties. The inflorescence is spadix-like, protogynous, 5-10 cm long, completely covered by peltate hexagonal scales.

\* Corresponding author.

Fagerlind (1938a, b) describes the distribution of flowers in the inflorescences of *Helosis*: the pistillate flowers are located in two rings around the axis of each scale; the staminate ones are on the periphery, under the corners of the scales. Staminate flowers of *Helosis* are characterized by a perianth with three apical lobes and an androecium with three fused stamens (Kuijt, 1969; Heide-Jørgensen, 2008). Erdtman (1966) and Hansen (1980a, b) describe pollen grains in *H. cayennensis*, *H. mexicana* and *H. guyanensis* as colpate and highlight the eurypalynous character.

No studies on the reproductive anatomy in the genus exist, except for a few details mentioned by Fagerlind (1938a, 1945) and Umiker (1920). Therefore, the aim of this study is to provide a complete description of the morphology and anatomy of the inflorescence and staminate flowers of *Helosis cayennensis* var. *cayennensis*, including the development and structure of the male gametophyte, in order to complete the embryological studies underway in American species of the Balanophoraceae family.

## MATERIAL AND METHODS

*Helosis* inflorescences were collected on the Apipé Grande Island, Ituzaingó department, province of Corrientes, Argentina. Voucher specimens are deposited in the Herbarium of the Botanical Institute of the Northeast, Corrientes (CTES; Gonzalez & Popoff No. 239, 18/12/2008).

Inflorescences at various developmental stages were fixed in FAA (formalin, 70% alcohol and acetic acid, 90:5:5). Samples were dehydrated according to the Johansen protocol (1940; modified by González & Cristóbal, 1997), and then embedded in paraffin. Transversal (TS) and longitudinal (LS) serial sections were cut 10-12 microns thick using an automated microtome. Sections were stained with safranin - fast green (Ruzin, 1999) or safranin - Astra blue (Luque & al., 1996). Lugol was used for identification of starch, FeSO<sub>4</sub> (Johansen, 1940) and IKI-H<sub>2</sub>SO<sub>4</sub> (Ruzin, 1999) for identification of tannins. Sections were analyzed using a Leica MZ6 stereomicroscope, Leica DMLB2, and Leica DM1000 optical and fluorescence microscope (LM), all provided with camera lucida and digital camera. Polarized light was additionally used to locate crystals and starch.

For analysis of flowers with a scanning electron microscope (SEM), the fixed in FAA material was dehydrated in acetone ascending series, dried by critical point in CO<sub>2</sub> and metalized with gold-palladium. SEM analyses were performed at the Electron Microscopy Service of the Universidad Nacional del Nordeste, Corrientes, using a microscope Jeol LV 5800-20 Kv.

Pollen grains were processed via an acetolysis technique (Erdtman, 1966). For LM analyses, grains were mounted on gelatin-glycerin (Johansen, 1940). Measurements of polar axis (P), equatorial diameter (E), area, colpus length, and thickness of exines were taken for 25 pollen grains. We calculated the minimum, maximum, and average of each parameter studied. Permanent preparations were deposited in the Herbarium Palinoteca CTES (PAL No. 2907). For SEM analyses temporary samples were mounted in a solution of distilled water and absolute alcohol (1:1) on aluminum foil, dried at room tem-

perature and coated with gold. The terminology used for pollen morphology is taken from Punt & al. (1994, 2007).

## RESULTS

*Helosis* has oval spadix-like inflorescences, lacking the spathe typical of a true spadix (Fig. 1 A-G). They are located on a fragile peduncle. Young inflorescences are covered with tightly arranged scales that are peltate, capitate, and hexagonal in front view (Fig. 1 A). The scales become black and fall at anthesis, exposing the inflorescence surface densely covered by a cushion of pink trichomes or filariae (Fig. 1 B, C). The pistillate flowers are the first to develop, in acropetal mode, exposing their stigmas over the layer of filariae (Fig. 1 C). When the stigmas fall off, the filariae change from pink to light brown, and staminate flowers appear (Fig. 1 D).

Each staminate flower consists of a 3-lobed deep pink tubular perianth and a synandrium formed by three stamens (Fig. 1 E, F, H). The inflorescences are constantly visited by ants (Fig. 1 F, arrows), but no secretion was observed. After male flowers fall off, the whole inflorescence acquires a brownish color and the inflorescence axis bends (Fig. 1 G).

### *Anatomy of inflorescences*

The inflorescence develops endogenously, the primordium is covered by a volva formed by two to four layers of tanniferous parenchyma (Fig. 2 A), and this tissue is an extension of the outer layers of the rhizome. As the inflorescence elongates, this tissue brakes apart and the rests remain at the inflorescence base.

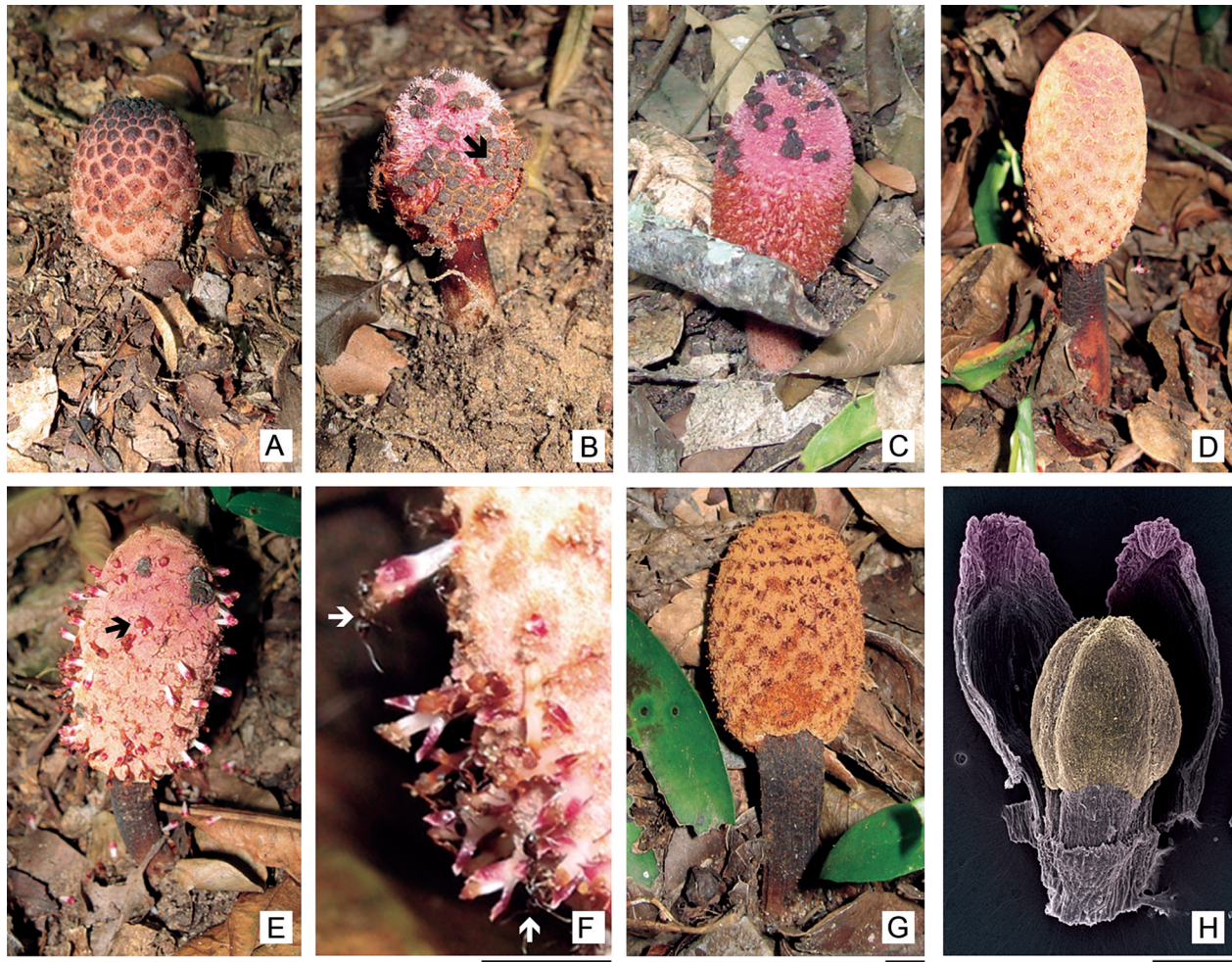
The peduncle lacks true epidermis; its surface is formed by a variable number of layers of parenchyma with thin walls and without intercellular spaces. The cytoplasm is tanniferous and remarkably dense. The outermost layers of the protective tissue become centripetally disorganized; cells lose contact and detach (Fig. 2 B). Cuticle, stomata or trichomes were not observed.

The peduncle central region has numerous vascular bundles dispersed in a matrix of parenchyma, but there is no cortex or medullae (Fig. 2 B). Parenchymatic cells are tanniferous with conspicuous nuclei and abundant simple starch grains with stellate fissures (Fig. 2 C). The vascular system is composed by numerous collateral vascular bundles, disposed disorderly (Fig. 2 B, D). The xylem is formed by short vessels with simple perforation plates; the walls have ingrowths toward the lumen cell (Fig. 2 E). The phloem has sieve tubes with composed sieve plates and abundant parenchymatic cells with conspicuous nuclei (Fig. 2 F).

The peltate scales that cover the young inflorescence lack an epidermis (Fig. 2 G, H). These scales consist of compact tanniferous parenchyma with thin walls and possess an unbranched vascular bundle. As the scales mature, the external tissues collapse centripetally.

The inflorescence surface lacks an epidermis and is totally covered by filariae (Figs. 1 B, C, 2 I, J). Filariae are multicellular trichomes 1.8-2.1 mm long, formed by 3-4 rows each consisting of 35-50 cells. These cells have a conspicuous nucleus and small starch granules. At the trichome the proximal cells are elongated and have tanniferous cytoplasm. Distal cells





**Fig. 1.** Inflorescences of *Helosis cayennensis* var. *cayennensis*. **A-G**, inflorescences. **A**, young inflorescence covered with peltate scales. **B, C**, inflorescence showing pink trichomes. **D**, inflorescence without peltate scales initiating anthesis of staminate flowers. **E**, inflorescence with synandria and perianths with pink lobes (arrow) emerging. **F**, staminate flowers with ants (arrows). **G**, brown ripe flower head. **H**, detail of the synandrium, one tepal was removed (SEM). Scale bars: F = 0,5 cm, G = 1 cm (also corresponds to figs. A-E), H = 20  $\mu$ m.

are vacuolated, rounded and give the moniliform appearance to the trichome apex (Fig. 2 I). Trichome cell walls are cellulosic and thin, the contact walls between rows of cells are undulated (Fig. 2 K, L). The trichome apical portion has a detached and finely striated cuticle (Fig. 2 J-M).

The matrix of the inflorescence axis has similar anatomical features as that of the peduncle: a tanniferous parenchyma and vascular bundles that innervate both scales and flowers. In a mature spadix cross-section, more than one hundred vascular bundles were found.

#### *Staminate flowers*

Staminate flowers are embedded in the dense layer of filariae from which they emerge as pollen matures (Fig. 2 N). The three tepals are fused at the base; each tepal is bilayered with cellulosic cell walls, tanniferous cytoplasm, and scarce starch grains. In the free apical portion of each tepal, the inner layer is differentiated into thick-walled, sclerosed cells; there are no stomata (Fig. 4 A). Each staminate flower has a conoidal inferior pistillode, formed by a compact parenchy-

ma without vascularization or female gametophyte differentiation (Figs. 2 N, 3 A, B).

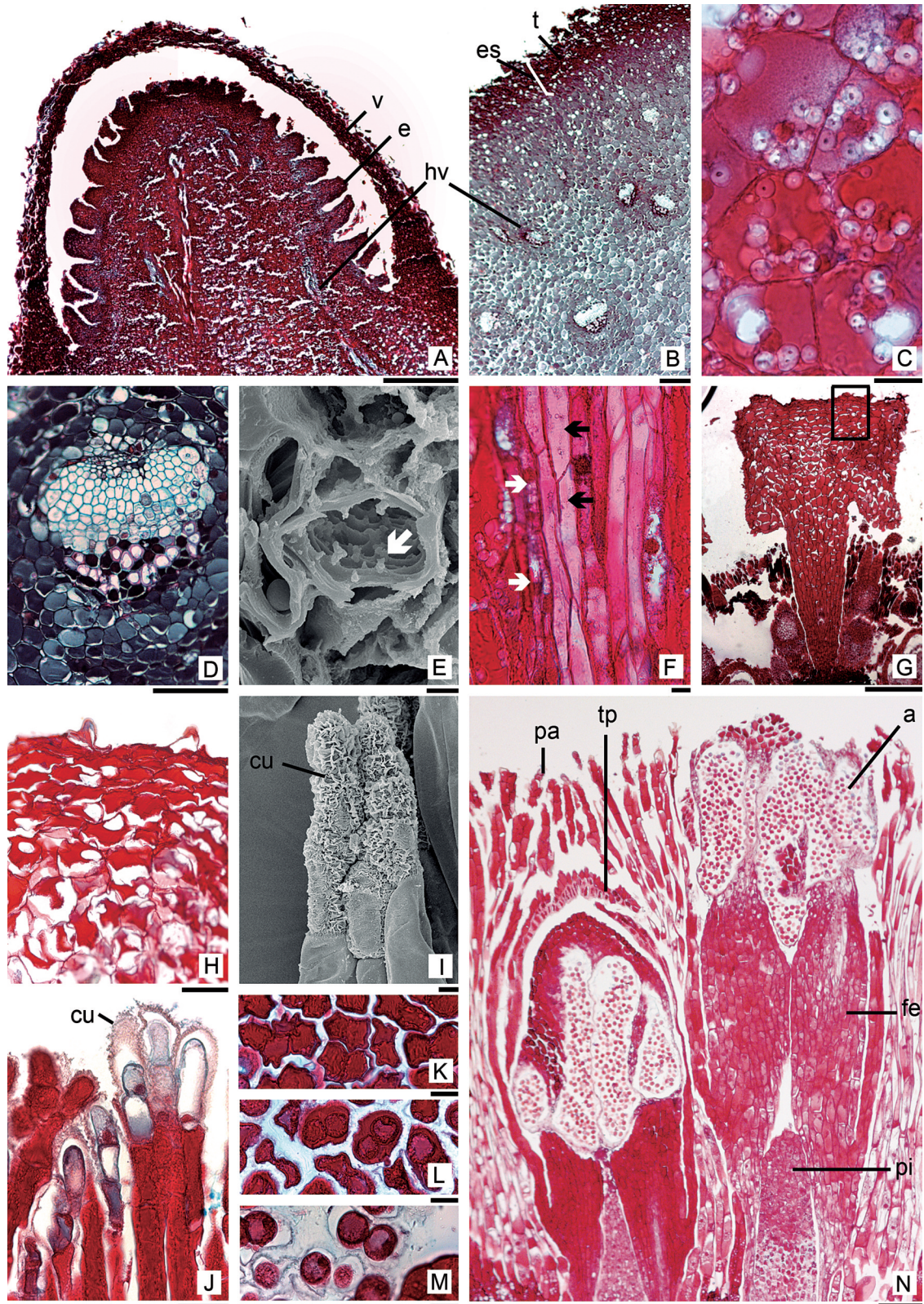
The three staminal filaments have a quasi-triangular section, they are welded together at their inner vertices (Fig. 3 B, C) and are shortly free at its most distal part. The filaments consist of tanniferous parenchyma and vascular bundles identical to those described for the axis of the inflorescence. Anthers are welded along their length. In transection the synandrium has nine pollen sacs. Six outer pollen sacs are arranged in pairs, located laterally to each filament, and the three inner pollen sacs alternate with the outer ones (Figs. 3 H, 4 D). In longitudinal view, the inner pollen sacs are longer than the outer ones (Figs. 2 N, 3 A).

The staminate flowers are vascularized by three bundles that derive directly from the inflorescence axis and innervate each staminal filament without branching (Fig. 3 A-H).

#### *Microsporogenesis and microgametogenesis*

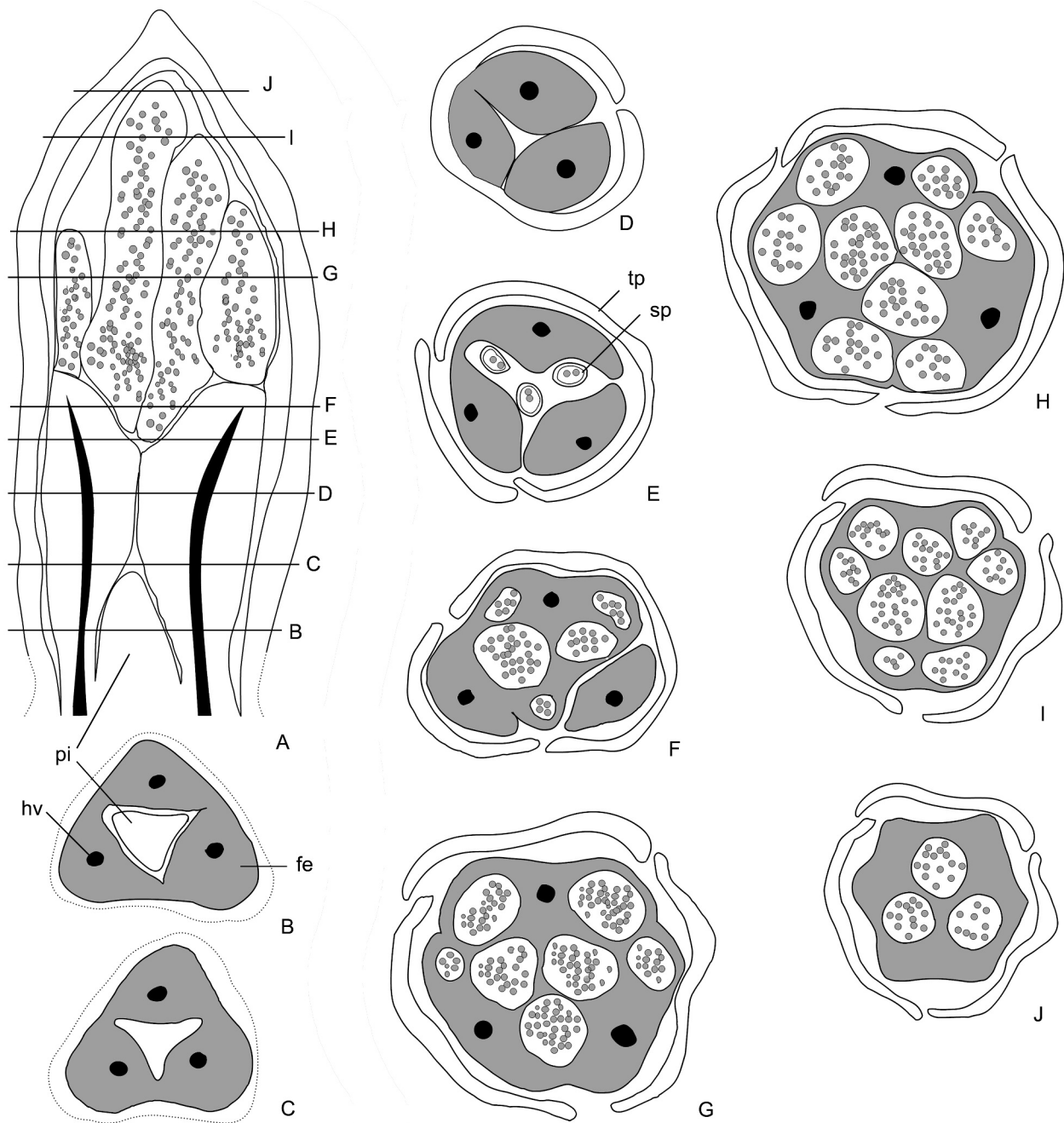
Sporogenous tissue completely fills the volume of the anther locules (Fig. 4 D). Cells are characterized by large, con-





**Fig. 2.** Inflorescences and flowers of *Helosia cayennensis* var. *cayennensis*. **A**, LS of a young inflorescence covered by volva and already formed scales; flowers absent. **B**, TS of the peduncle showing external protective layers, tanniferous parenchyma matrix and vascular bundles. **C**, details of tanniferous parenchyma with starch grains. **D**, TS of a vascular bundle. **E**, (SEM) vessel member with ingrowth (arrow). **F**, LS of a vascular bundle showing sieve tubes (black arrows) and parenchyma cells (white arrows). **G**, LS of a scale. **H**, detail of surface of the ripe scale (region indicated by a square in H). **I-M**, filariae. **I**, (SEM) detail of filariae apex, striated cuticle. **J**, LS of filariae. **K-M**, TS of filariae at the proximal (K), medial (L) and distal part (M). **N**, LS of young staminate flowers (left) and mature one at anthesis (right), both embedded in layer of filariae. Abbreviations: a: anther, cu: cuticle, e: scales, es: surface layers, fe: staminal filament, hv: vascular bundles, pa: filariae, pi: pistillode, tp: tepals, v: volva. Scale bars: A, G = 500  $\mu$ m, B = 200  $\mu$ m, C, E, F, I, K-M = 20  $\mu$ m, D, N, H = 100  $\mu$ m, J = 50  $\mu$ m.





**Fig. 3.** Scheme of the floral vascularization of a staminate flower in *Helosis cayennensis* var. *cayennensis*. **A**, Longitudinal view indicating the levels of the cross sections in B-J. **B-D**, staminal filament. **E-J**, anther tissue. Abreviaturas: fe: staminal filament, hv: vascular bundles, pi: pistillode, sp: pollen sacs, tp: tepals.

spicuous nuclei and one or two nucleoli, and the cytoplasm is very dense (Fig. 4 E). The shape of these cells change from polygonal to rounded, and cells separate from each other and from the tapetum to become microspore mother cells (MMC; Fig. 4 G). Meiosis is initiated simultaneously; MMCs are surrounded by a callose wall (Fig. 4 H). Meiosis takes place in the usual way, thin chromosomes appear during prophase I; chromosomes acquire their maximum condensation and are located in the equatorial plane in the metaphase I; they move to the poles, and the CMM reach anaphase I forming a dyad of cells enclosed in a callose wall (Fig. 4 H-K). Meiosis II occurs rapidly and cytokinesis forms tetrads simultaneously, the

four microspore are surrounded by callose walls (Fig. 4 L, arrow). The tetrads are tetrahedral (Fig. 4 M).

When the callose wall disappears, the microspores are separated and gradually change their shape from polygonal to rounded. A very dense cytoplasm remains; the nucleus occupies a parietal position and divides by mitosis (Fig. 4 N, O). Progressively, the wall cells become thickened, and the generative cell nucleus splits resulting in a pollen grain with a vegetative nucleus and two voluminous and lenticular generative nuclei (Fig. 4 D). The microgametogenesis is completed with the formation of tricellular pollen grains before the dehiscence of the anthers.



### Anther wall

At premeiotic state, the anther wall consists of the epidermis, one or two parietal layers and the tapetum, and all cells are characterized by large nuclei and thin cellulosic walls (Fig. 4 E, F). No cuticle or stomata were observed in the epidermis. At the state of free microspores, the parietal layers collapse while tangentially crushed (Fig. 4 G, H). The tapetum is of secretory type and is formed by a layer of large uninucleate cells, with very dense cytoplasm; the internal tangential wall is distended, so that the cells occupy much of the locule volume (Fig. 4 E-J). Tapetal cells retain their conspicuous nuclei and cellular integrity during microsporogenesis, but degenerate rapidly after the first mitosis of pollen grains (Fig. 4 K).

During anther development, endothecium or mechanical layers are not formed. At the three-cellular state of pollen grains, the synandrium emerges from the mass of trichomes, due to elongation of the filaments (Fig. 2 N). Dehiscence occurs by formation of three apical longitudinal grooves that join at the apex and open towards the base of the synandrium (Fig. 5 A). Dehiscence is caused by lysis of the epidermis, the parietal layers, and the remains of the tissue that connects the paired outer pollen sacs (Figs. 4 P, 5 A). The layers that separate the inner and outer pollen sacs also collapse.

### Pollen morphology

Pollen grains are 3-colpate, sometimes 4-colpate, isopolar and radially symmetrical, small  $P = 23$  (29.7)  $31 \mu\text{m}$ ,  $E = 23$  (30.7)  $32 \mu\text{m}$ . In equatorial view they appear spheroidal  $P/E = 92$  (118)  $124$  (Fig. 5 C). Colpi are thin (3-4  $\mu\text{m}$ ) and long with rounded edge, and subcircular in polar view (Fig. 5 B, E-G). The apocolpium is 4-5  $\mu\text{m}$  thick. The exine is 1  $\mu\text{m}$  thick, whereas the sexine and nexine are undifferentiated. The tectum is psilate. SEM analyses revealed that the exine structure is of the granular type (Fig. 5 D), confirming that it is tectate and finely rugulate (Fig. 5 B).

## DISCUSSION AND CONCLUSIONS

### Sterile parts of the inflorescence

Of the 17 Balanophoraceae genera six are grouped in the Helosiodoideae subfamily: *Helosis*, *Corynaea*, *Ditepalanthus*, *Exorhopala*, *Scybalium*, and *Rhopalocnemis* (Eberwein, 2000; Nickrent, 2002; Takhtajan, 2009). The presence of peltate scales falling from the inflorescence before anthesis is a characteristic of the genera in this subfamily. The scales of *Helosis* have a simple anatomical structure, as they consist of a compact parenchyma with abundant tannins and starch and are vascularized by a single vascular bundle. Engell (1979) found an identical structure in the scales of *Corynaea crassa*. A peculiarity observed in *Helosis*, is that its scales lack a true epidermis, and the scale surface is instead formed by several cell layers, which gradually collapse forming a protective covering. Presence of such a protective coating instead of the true epidermis was also observed on the peduncle of the inflorescence.

Sterile portions of *Helosis* inflorescences display anatomical features observed in the vegetative body of several holoparasites: replacement of the epidermis by a multilayered protective tissue, almost universal presence of tanniferous

cells and development of ingrowths in tracheary elements. Such peculiar features had already been described for the vegetative body of *Helosis* by Hsiao & al. (1993) and Shu-Chuan & al. (1993), as well as in other holoparasites, including *Lophophyton leandrii* and *L. mirabile* (González & Mauseth, 2010; Sato & González, 2013) and *Ombrophytum subterraneum* (Mauseth & Montenegro, 1992).

The filariae or paraphyses that cover the inflorescence of Helosiodoideae subfamily and surround the flowers are considered nectar-secreting structures by Heide-Jørgensen (2008). In *Rhopalocnemis*, these structures are indeed interpreted as nectariferous by van Steenis (1931), due to the secretion and scent they produce. Engell (1979) also came to similar conclusions about the filariae in *Corynaea crassa* after studying their anatomy using inflorescences fixed in a liquid preservative. The filariae of *Helosis* have an identical anatomy to *C. crassa*, in which the distended cuticle in the apical portion would indicate their secretory nature. Although *Helosis* living inflorescences had no secretion or scent (detectable by humans), they were covered with ants, suggesting that the absence of nectar could be explained by the fact that the ants remove the nectar as it is secreted.

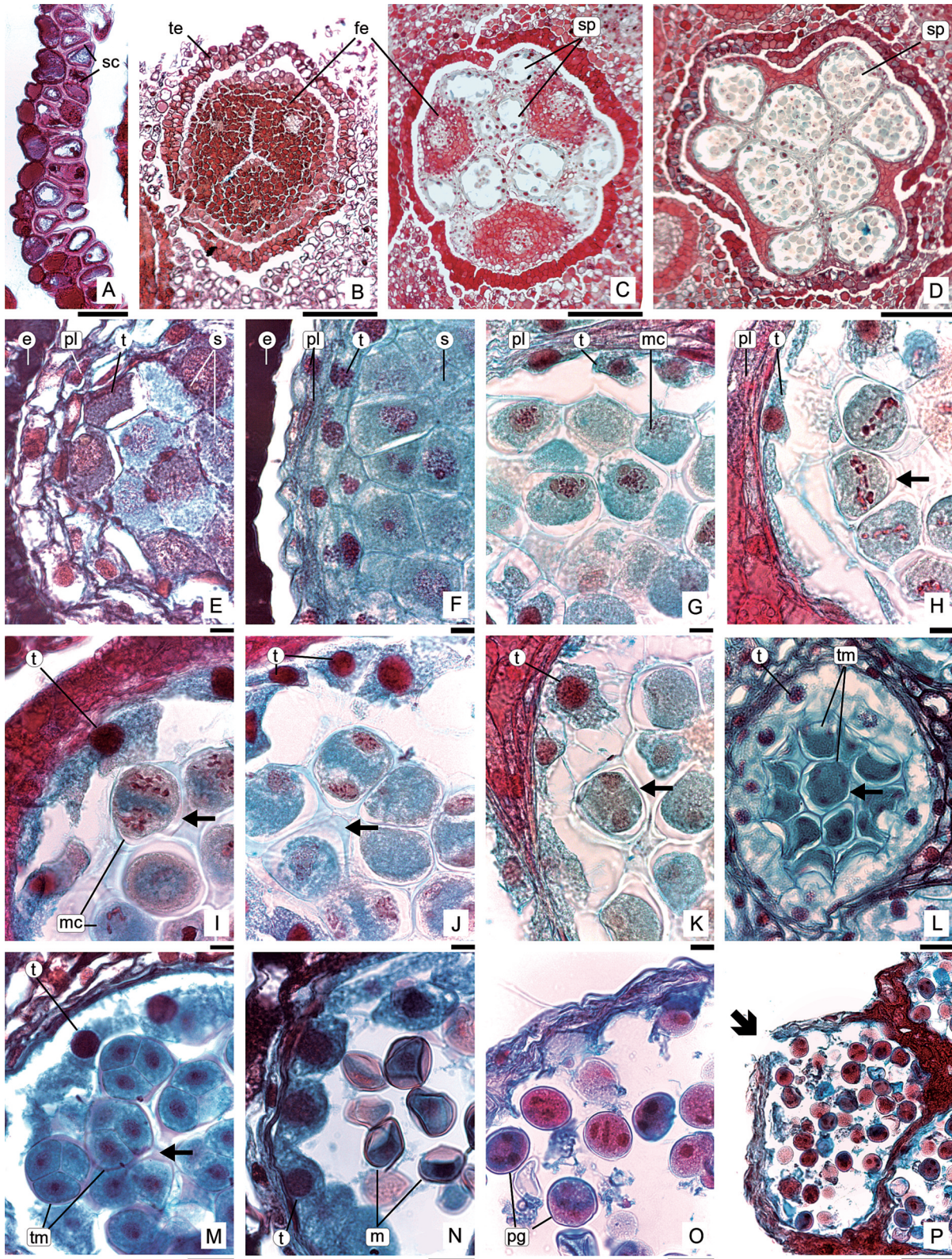
### Flower

Balanophoraceae is one of the eudicots families characterized by trimerous flowers (Endress, 1996). The presence of an irregularly lobed perianth is restricted to male flowers of subfamily Helosiodoideae (Harms, 1935). According to Fagerlind (1938a), the tepals of *Rhopalocnemis* and *C. crassa* are fused, but in the latter species perianth segments are free (as observed by Engell, 1979, too). In *H. cayennensis* (this study) and *Exorhopala ruficeps* (Eberwein & Weber, 2004), tepals are fused for a short part at the base. The presence of an inner epidermis formed by sclereids in *H. cayennensis* is consistent with the description of the perianth of *C. crassa*.

The synandrium is a characteristic of subfamily Helosiodoideae (Umiker, 1920; Kuijt, 1969; Engell, 1979; Eberwein & Weber, 2004). Studies on the flowers vascular supply in the subfamily show that the androecium always consists of three stamens but there are discrepancies in the number of pollen sacs among genera or even within a same species. In *E. ruficeps*, Eberwein & Weber (2004) described trimerous staminate flowers with anthers fused into a globose synandrium consisting of 10 to 16 pollen sacs. These authors consider that the inner pollen sacs do not correspond to any particular stamen, and, therefore, transferred *E. ruficeps* to *Helosis ruficeps*. Engell (1979)'s description of staminate flowers of *C. crassa* agrees with that of *Helosis* staminate flowers studied here but *C. crassa* flowers have only three to six pollen sacs. *Helosis* flowers have three stamens, each with its own vascular bundle; nevertheless they have nine pollen sacs, in which the three inner ones alternate with the outer ones, making it difficult—as in *H. ruficeps*—to assign with certainty which pollen sac pertains to which stamen.

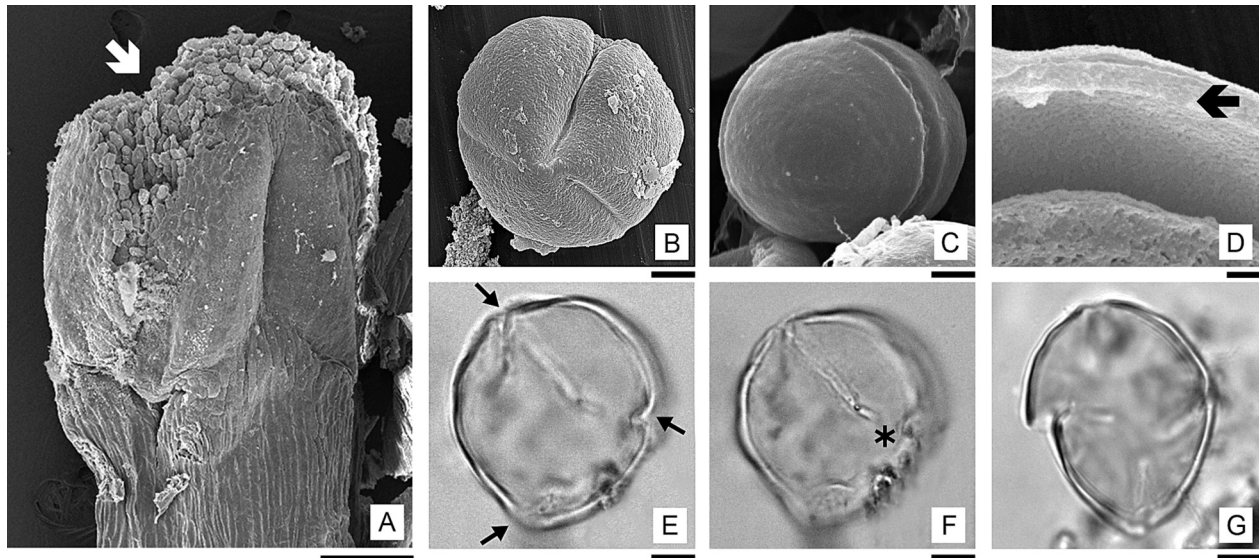
Presence of a synandrium and stamens reduced to an undefined or variable number of pollen sacs is frequently observed in families with flowers with a high level of reduction, as in Araceae (Grayum, 1990) and Myristicaceae (De Wilde, 2000; Sauquet, 2003). This reduction is a character common in par-





**Fig. 4.** Microsporogenesis and microgametogenesis of *Helosis cayennensis* var. *cayennensis*. **A**, TS of the inner epidermis of a tepal with sclerosed cells (arrows). **B-D**, TS of the synandrium from proximal to distal levels of a bud, **B**, staminal filament, **C**, pollen sacs base, **D**, medial zone of the anther. **E-O**, TS of the pollen sacs displaying microsporogenesis and microgametogenesis. **E**, sporogenous and tapetum cells. **F**, early microsporocyte stage. **G**, microsporocytes at meiosis, early prophase I. **H**, metaphase I. **I**, anaphase I. **J**, telophase I. **K**, dyad cells enclosed in a callose layer with tapetum cells around. **L**, meiosis II, tetrahedral tetrads. **N**, microspores. **O**, bicellular pollen grains. **P**, pollen sacs showing anther dehiscence zone (arrow) and tricellular pollen grains. Abbreviations: e: epidermis, fe: estaminal filaments, mc: microspore mother cells, m: microspore, pg: pollen grains, pl: parietal layers, s: sporogenous tissue, sp: pollen sacs, sc: sclerosed cells, t: tapetum, te: tepals, tm: tetrads of microspores, callose: thin arrows. Scale bars: A, P = 50  $\mu$ m, B-D = 200  $\mu$ m, E-K = 10  $\mu$ m, L-O = 20  $\mu$ m.





**Fig. 5.** Synandrium dehiscence and pollen grains of *Helosis cayennensis* var. *cayennensis*. **A**, SEM microphotograph of synandrium apex showing apical slit. **B-G**, photographs of pollen grains. **B-D**, SEM: **B**, polar view. **C**, equatorial view of the pollen grain without acetolysis. **D**, exine section (arrow) showing the granulate structure. **E-G**, LM cross sections. **E**, pollen grain 3-colpate (arrows) in polar view. **F**, superficial view, apocolpi (\*). **G**, pollen grain 4-colpate. Scale bars: A = 20  $\mu\text{m}$ ; B, C, E-G = 10  $\mu\text{m}$ , D = 1  $\mu\text{m}$ .

asitic plants, for example *Korthalsella* possess reduced flowers (Viscaceae, Huaxing & Gilbert, 2003), or in *Balanophora*, *Langsdorffia*, *Thonningia* and *Rhopalocnemis* genera (Balanophoraceae, Kuijt, 1969; Eberwein & al., 2009). In Apodanthaceae, the anthers form a tube that surrounds the sterile gynoecium, in which neither thecae nor stamens can be recognized, pollen sacs are arranged in pairs, and the dehiscence is produced by a transverse aperture through the two rings of the pollen sacs (Blarer & al., 2004).

#### *Androecium anatomy and pollen morphology*

Analyses of the floral vascularization suggest an extreme reduction of *Helosis* flowers. Indeed only the stamens possess vascular supply (which originates directly from the inflorescence axis), whereas, the perianth and the pistillode lack any vascularization.

Anther walls in *Helosis* possess an epidermis, parietal layers, and a secretory tapetum. Umiker (1920) describes *Helosis* anthers having a plasmodial tapetum and tetrahedral tetrads formed by simultaneous cytokinesis. He postulates that *Helosis* is an apomictic plant and observed no endothecium or other indication of anther dehiscence. The endothecium is indeed absent also in other parasitic plants, as *Ditepalanthus* (Fagerlind, 1938b), *Balanophora* (Fagerlind, 1945), and *Pilostyles* (Rutherford, 1970). However, our observations indicate that staminate flowers emerge from the mass of filariae by the growth of staminal filaments, so that the pollen sacs are exposed to atmosphere; the synandrium dehiscence occurs through apical openings without mechanical layers; pollen is released to tricolpate state. These observations are consistent with those of Lotsy (1901) for *Rhopalocnemis*. Engell (1979) describes the same anatomy in anthers of *C. crassa*, he mentions that some male flowers do not emerge from the mass of filariae, and probably do not release pollen (but these could have been young flowers, as it occurs in *Helosis*).

Maybe one of the earliest works on apical dehiscence is that of Harris (1905), who classified them into seven types and already mentions Balanophoraceae alongside the Araceae in type 1 or "Araceous Type", often characterized by anthers united in one synandrium. Dehiscence through apical openings without endothecium is a common feature in flowers with stamens attached at synandrium (Maheshwari, 1950; Eames, 1961; Esau, 1965; Davis, 1966). However, later studies showed that this correlation cannot be generalized and even is wrong in monocots with poricidal dehiscence (Gerenday & French, 1988) or in families like Begoniaceae (Tebbit & Maciver, 1999).

The staminate flowers of *Helosis* have a rudimentary gynoecium; totally inferior. In *H. ruficeps*, Eberwein & Weber (2004) described staminate flowers without rudimentary gynoecium, however it is evident in the photographs of the inflorescence. Of the related genera, their presence was described in *C. crassa* (Engell, 1979).

*Lophophytum* is another genus of Balanophoraceae where the anatomy and development of the male gametophyte was studied (Sato & González, 2013), but belongs to the subfamily Lophophytoideae (Takhtajan, 2009) in which the inflorescences lack filariae. In *Lophophytum* the stamens are free and have a longitudinal dehiscence with normal stomium and endothecium.

Poricidal dehiscence in *Helosis* flowers may represent an adaptation to the presence of the dense mass of filariae that could hinder pollen release. Pollen is in fact released when the anthers emerge and are exposed above the dense layer of filariae.

Angiosperm pollen can be classified according to the number of nuclei into binucleate (the most frequent) or trinucleate (Brewbaker, 1967). *Helosis* has trinucleate pollen grains, as have all other genera of the subfamily (Takhtajan, 2009). Pollen morphology (size, shape, number and type of apertures) in *Helosis cayennensis* described here agrees with that

of *H. guyanensis* and *H. mexicana* described by Hansen (1980b) and Erdtman (1966). Palynological studies in Helosidoideae showed that *Helosis*, *Corynaea*, *Ditepalanthus*, and *Rhopalocnemis* have the same pollen type: isopolar, 3-colpate, medium sized (less than 40 µm), and with rugulate exine (Erdtman, 1966; Hansen, 1980b). However, *Scybalium* has a different pollen type: apolar, 6(7-8)-pororate, large (more than 40 µm), and with psilate exine (Hansen, 1980b).

In conclusion, despite the extreme reduction of flowers, the anatomic characteristics and gametophyte development of staminate flowers of *Helosis cayennensis* are perfectly normal and functional. They are thus highly similar to other genera of the holoparasitic subfamily Helosidoideae. Sterile parts of flowers and inflorescence maintain the same distinctive and aberrant features of the plant vegetative parts.

## ACKNOWLEDGEMENTS

We thank to Binational Yaciretá Entity for their cooperation to collect the plant material. The authors also thank Prof. Brigitte Marazzi for improving the English language. This work was performed with grants from the Universidad Nacional del Nordeste (PICTO 199-2011 and PI-SGCyT 12P001).

## REFERENCES

- Blarer, A., Nickrent, D.L. & Endress, P.K. 2004. Comparative floral structure and systematic in Apodanthaceae (Rafflesiales). *Plant Systematic and Evolution* 245: 119-142.
- Brewbaker, J.L. 1967. The distribution and phylogenetic significance of binucleate and trinucleate pollen grains in the angiosperms. *American Journal of Botany* 54: 1069-1083.
- Davis, G.L. 1966. *Systematic embryology of the angiosperms*. J. Wiley, New York.
- De Wilde, W.J.J.O. 2000. Myristicaceae. *Flora Malesiana, Series I* 14: 1-632.
- Eames, A.J. 1961. *Morphology of the angiosperms*. McGraw-Hill, New York.
- Eberwein, R.K. 2000. Morphologie und Taxonomie der Helosidoideae (Balanophoraceae). *Linzer Biologische Beiträge* 32: 615-616.
- Eberwein, R.K. & Weber, A. 2004. *Exorhopala ruficeps* (Balanophoraceae): morphology and transfer to *Helosis*. *Botanical Journal of the Linnean Society* 146: 513-517.
- Eberwein, R., Nickrent, D.L. & Weber, A. 2009. Development and morphology of flowers and inflorescences in *Balanophora papuana* and *B. elongata* (Balanophoraceae). *American Journal of Botany* 96: 1055-1067.
- Endress, P. 1996. Homoplasy in Angiosperm flower. In: Sanderson, M.J. & Hufford, L. (eds.), *Homoplasy: The Recurrence of Similarity in Evolution*. Academic Press.
- Engell, K. 1979. Morphology and embryology of Scybalioidae (Balanophoraceae): 1. *Corynaea crassa* Hook. f. var. *sprucei* (Eichl.) B. Hansen. *Svensk Botanisk Tidskrift* 73: 155-166.
- Erdtman, G. 1966. *Pollen morphology and plant taxonomy Angiosperms*. Hafner Publishing Company, New York and London.
- Esau, K. 1965. *Plant anatomy*, 2d ed. John Wiley, New York.
- Fagerlind, F. 1938a. Bau und Entwicklung der floralen Organe von *Helosis cayennensis*. *Svensk Botanisk Tidskrift* 312.
- Fagerlind, F. 1938b. *Ditepalanthus*, eine neue Balanophoraceen-Gattung aus Madagaskar. *Arkiv för Botanik* 29 A. 24.
- Fagerlind, F. 1945. Blüte und Blütenstand der Gattung *Balanophora*. *Botaniska Notiser* 45: 330-350.
- Fontana, J.L. & Popoff, O.F. 2006. *Helosis* (Balanophoraceae) en Argentina. *Boletín de la Sociedad Argentina de Botánica* 41: 85-90.
- Gerenday, A. & French, J.C. 1988. Endothelial thickenings in anthers of porate Monocotyledons. *American Journal of Botany* 75: 22-25.
- González, A.M. & Cristóbal, C. 1997. Anatomía y ontogenia de semillas de *Helicteres lbotzkyana* (Sterculiaceae). *Bonplandia* 9: 287-294.
- González, A.M. & Mauseth, J.D. 2010. Morphogenesis is highly aberrant in the vegetative body of the holoparasite *Lophophytum leandrii* (Balanophoraceae): All typical vegetative organs are absent and many tissues are highly modified. *International Journal of Plant Science* 171: 499-508.
- Grayum, M.H. 1990. Evolution and Phylogeny of the Araceae. *Annals of the Missouri Botanical Garden* 77: 628-697.
- Hansen, B. 1980a. Pollen dimorphism in *Lophophytum mirabile* Schott & Endl. (Balanophoraceae). *Grana* 19: 189-191.
- Hansen, B. 1980b. Balanophoraceae. *Flora Neotropica, Monograph* 23: 1-80.
- Hansen, B. & Engell, K. 1978. Inflorescences in Balanophoroideae, Lophophytoideae and Scybalioidae (Balanophoraceae). *Dansk Botanisk Tidsskrift* 72: 177-187.
- Harms, H. 1935. Balanophoraceae. In: Engler, A. & Prantl, K. (eds.), *Die Natürlichen Pflanzenfamilien*, 296-339. Engelmann, Leipzig, Germany.
- Harris, J.A. 1905. The dehiscence of anthers by apical pores. *Annual Report of the Missouri Botanical Garden* 16: 167-257.
- Heide-Jørgensen, H.S. 2008. *Parasitic flowering plants*. Brill Academic Publishers, Leiden.
- Hsiao, S.C., Mauseth, J.D. & Gómez, L.D. 1993. Growth and anatomy of the vegetative body of the parasitic angiosperm *Helosis cayennensis* (Balanophoraceae). *Bulletin of the Torrey Botanical Club* 120: 295-309.
- Huaxing, Q. & Gilbert, M.G. 2003. Viscaceae. *Flora of China* 5: 240-245.
- Johansen, D.A. 1940. *Plant Microtechnique*. McGraw-Hill, New York.
- Kuijt, J. 1969. *The biology of parasitic flowering plants*. University of California Press.
- Lotsy, J.P. 1901. *Rhopalocnemis phalloides*, a morphological-systematic study. *Annales du Jardin Botanique de Buitenzorg* 2: 73-101.
- Luque, R.H., Sousa, C. & Kraus, J.E. 1996. Métodos de coloração de Roeser (1972)-modificado-e Kropp (1972) visando a substituição do azul de astra por azul de alcião 8 GS ou 8 GX. *Acta Botânica Brasileira* 10: 199-212.
- Maheshwari, P. 1950. *An introduction to the embryology of angiosperms*. McGraw-Hill, New York.
- Martínez y Pérez, J.L. & Rosas, R.A. 1995. Balanophoraceae. *Flora de Veracruz* 85: 1-7.
- Mauseth, J.D. & Montenegro, G. 1992. Secondary wall ingrowths on vessel elements in *Ombrophytum subterraneum* (Balanophoraceae). *American Journal of Botany* 79: 456-458.
- Mauseth, J.D., Hsiao, S.C. & Montenegro, G. 1992. Vegetative body of the parasitic angiosperm *Helosis cayennensis* (Balanophoraceae). *Bulletin of the Torrey Botanical Club* 119: 407-417.
- Nickrent, D.L. 2002. Orígenes filogenéticos de las plantas parásitas. In: López-Sáez, J.A., Catalán, P. & Sáez, L. (eds.), *Plantas parásitas de la península Ibérica e islas Baleares*. Madrid: Mundi-Prensa, 29-56.
- Punt, W., Blackmore, S., Nilsson S. & Le Thomas, A. 1994. *Glossary of pollen and spore terminology*. LPP Foundation, University of Utrecht.
- Punt, W., Hoen, P.P., Blackmore, S., Nilsson, S. & Le Thomas, A. 2007. Glossary of pollen and spore terminology. *Review of Paleobotany and Palynology* 143: 1-81.
- Rutherford, R.J. 1970. The anatomy and cytology of *Pilostyles thurberi* Gray (Rafflesiaceae). *Aliso* 7: 263-288.
- Ruzin, S.E. 1999. *Plant microtechnique and microscopy*. Oxford University Press.
- Sato, H.A. & González, A.M. 2013. Flores estaminadas en *Lophophytum*. *Boletín de la Sociedad Argentina de Botánica* 48: 59-72.
- Sauquet, H. 2003. Androecium diversity and evolution in Myristicaceae (Magnoliales), with a description of a new malagasy genus, *Doyleanthus* gen. nov. *American Journal of Botany* 90: 1293-1305.
- Shu-Chuan, H., Mauseth, J.D. & Gómez, L.D. 1993. Growth and anatomy of the vegetative body of the parasitic angiosperm *Helosis cayennensis* (Balanophoraceae). *Bulletin of the Torrey Botanical Club* 120: 295-309.
- Takhtajan, A.L. 2009. *Flowering plants*. Springer.
- Tebbit, M.C. & Maciver, C.M. 1999. The systematic significance of the endothecium in Begoniaceae. *Botanical Journal of the Linnean Society* 131: 203-221.
- Umiker, O. 1920. Entwicklungsgeschichtlich-cytologische Untersuchungen an *Helosis guyanensis* Rich. *Arbeiten des Instituts für Allgemeine Botanik und Pflanzenphysiologie der Universität Zürich* 23: 1-54.
- Van Steenis, C.G.G.J. 1931. Some remarks on the genus *Rhopalocnemis* Junghuhn. *Handelingen 6. Nederl.-Indisch Natuurwetenschappelijk Congres, Bandoeng, Java. Meded. Biol. Sectie* 464-475.

Associate Editor: M. Angélica Bello Gutiérrez

Received: 9-VII-2013

Accepted: 5-XII-2013